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Spore Morphology of the Polypodiaceae from Northwestern Argentina

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ABSTRACT.—The spores of the following genera of Polypodiaceae growing in northwest Argentina were analyzed: *Campyloneurum*, *Microgramma*, *Pecluma*, *Phlebodium*, *Pleopeltis* and *Polypodium*. The study involved analyses of herbarium material using light microscopy and scanning electron microscopy. The spores are monolete, 40–90 μm in major equatorial diameter, eliptic to oblong in polar view and plane to concave-convex in equatorial view. The exospore ranges from 2–5 μm thick, is apparently double-layered, with a verrucate or tuberculate surface that is usually perforated. The perispore ranges from 0.3–1 μm thick, is apparently single-layered, attached to the exospore, perforated, and generally smooth or in some cases micro-ornamented. Most of the taxa analyzed have globules on the surface. These are single or associated in masses and irregularly distributed. Characteristics such as size, shape and exospore and perispore sculpture allow us to differentiate among some of the genera as well as recognize species groups. *Microgramma*, *Campyloneurum*, *Pecluma*, *Pleopeltis* and *Polypodium* have verrucate spores whereas those of *Phlebodium* are tuberculate.

This study forms part of a project dealing with the palynological flora of Northwest Argentina. According to de la Sota (1973), this region comprises the provinces of Jujuy, Salta , Tucumán, Catamarca, the eastern part of La Rioja, and southwestern Santiago del Estero (Fig. 1).

The following members of Polypodiaceae grow in this region: *Polypodium argentinum* Maxon, *P. bryopodum* Maxon, *P. chrysolepis* Hook., *P. lasiopus* Klotzsch, *P. loriceum* L., *P. pleopeltidis* Fée, *P. squalidum* Vell., *P. tweedianum* Hook., *Campyloneurum aglaolepis* (Alston) de la Sota, *C. lorentzii* (Hieron.) Ching, *C. major* (Hieron. *ex* Hicken) Lellinger, *C. tucumanense* (Hieron.) Ching, *Microgramma squamulosa* (Kaulf.) de la Sota, *Pecluma filicula* (Kaulf.) M. G. Price, *P. oranense* (de la Sota) de la Sota, *P. venturi* (de la Sota) M.G. Price, *Phlebodium pseudoaureum* (Cav.) Lellinger and *Pleopeltis macrocarpa* (Bory ex Willd.) Kaulf. (de la Sota, 1960, 1977; Ponce,1996). *Polypodium loriceum L.* recently collected by Martínez and de la Sota, (Sota, et al. 1999) in Salta province, was also included in the study. According to Ponce (1996), *Polypodium hirsutissimun* probably grows in the region, however, no material documenting its occurrence was found in the herbaria. According to Tryon and Tryon (1982) and Tryon and Lugardon (1991), American Polypodiaceae are mostly diploid.

The spores of the Polypodiaceae have been described and illustrated with LM by Nayar and Devi (1964), Lloyd (1981) and Pal and Pal (1970), with SEM

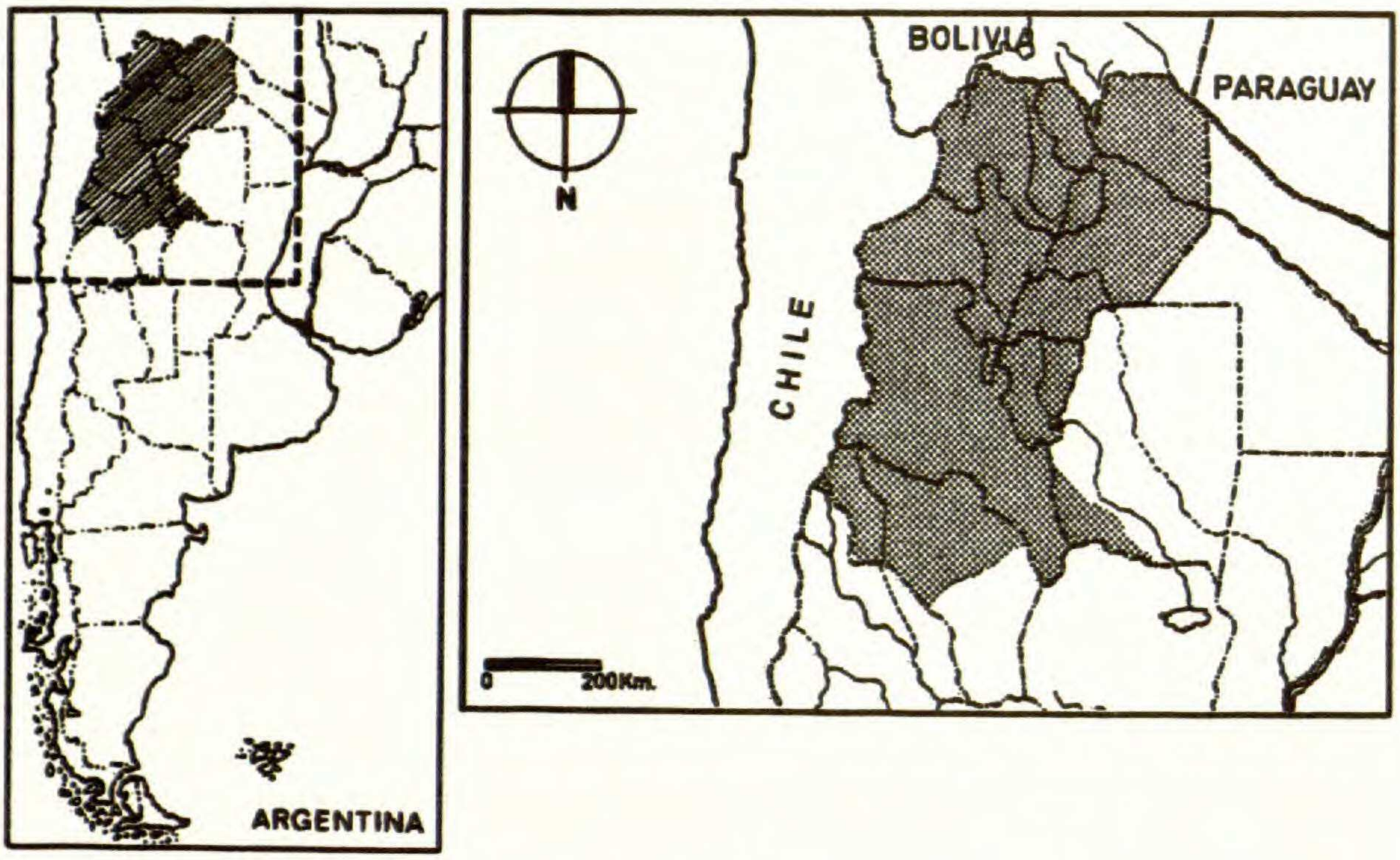

FIG. 1. Study area (Northwest Argentina).

and TEM by Tryon and Lugardon (1991), Tryon and Tryon (1982), van Uffelen (1992, 1993, 2000), van Uffelen and Hennipman (1985) and Hennipman (1990). However among these contributions there are not many references to the spores of species that grow in Argentina.

Tryon and Tryon (1982) differentiated 6 informal groups of *Polypodium* species from tropical America. Those groups were based on a combination of characters such as stem scales, lamina indument, venation, soral arrangement and spores.

The aim of this study is to analyze the spores of the Polypodiaceae that grow in northwestern Argentina in order to add to the existing information about these taxa and to assess the systemic value of palynological data.

MATERIALS AND METHODS

Spores were obtained from herbarium (BA, LP, LIL and SI) specimens and were studied using light (LM) and scanning electron microscopy (SEM). For LM the spores were treated with hot 3% sodium carbonate for 2 minutes and acetolyzed according to the method of Erdtman (1960). For SEM, the material was treated with hot 3% sodium carbonate, washed, dehydrated, suspended in 96% ethanol and then transferred to acetate plates. After dryng they were coated with gold. Wall fractures obtained using ultrasound for 10 minutes were also used in order to study the sporoderm structure. All the observations were made with Olympus BH2 and BHB light microscopes and a JEOL JSMT- 100 scanning electron microscope at the Museo de Ciencias Naturales de La Plata.

The terms proposed by Hennipman (1990), Tryon & Lugardon (1991), van Uffelen & Hennipman (1985) and van Uffelen (1993) were used for spore descriptions.

Spores were characterized for: color of acetolyzed material, shape, diameters, laesurae, sporoderm thickness, and ornamentation, structure and stratification of the wall layers. In some species of *Campyloneurum*, the number of spores produced per sporangium was considered in order to understand spore irregularities related to size and morphology.

The letters MP, associated with the list of specimens investigated (Table 1) indicate the reference number of each palynological sample as filed in the Laboratorio de Palinología, Facultad de Ciencias Naturales y Museo de La Plata.

Results

Campyloneurum (Table 2; Fig. 2, A–L)

This genus is represented by four species in the study area: *C. aglaolepis* (Alston) de la Sota, *C. lorentzii* (Hieron.) Ching, *C. major* (Hieron. ex Hicken) Lellinger and *C. tucumanense* (Hieron.) Ching. All are epiphytic and characterized by an entire lamina, anastomosing veins and round sori borne in a marginal or submarginal position.

Campyloneurum tucumanense is the largest species of the genus in the northwestern of Argentina. The lamina is soft, membranaceous and has the most complex and evident venation of the *Campyloneurum* studied. Ponce (1996) considered this species to be endemic to the region of study although it has also been reported for Bolivia by Lellinger (1988).

The spores are ellipsoidal or oblong in polar view (Fig. 2 A, D and J) and plane to concave-convex in equatorial view (Fig. 2 B, F, G and K) . In *Campyloneurum aglaolepis*, *C. lorentzii*, and *C. major*, equatorial diameters range between 70 and 80 μm and polar diameters between 40 and 60 μm. In *Campyloneurum tucumanense* the equatorial diameter ranges between 85 and 94 μm and 46–60 μm in polar diameter.

The exospore is the thickest wall layer, ranging between 1.5 and 3 μm in all species except in *Campyloneurum tucumanense* in which it reaches 4 μm. Spores are verrucate with a dense compact wall (Fig. 2 I). With LM it is apparently double-layered in section with a compact structure. The inner layer (ie) is thiner than the outer (ie: oe ratio 1:2–1:3). The outer layer (oe) forms the elements of the sculpturing (verrucae). The verrucae have a circular or polyhedral outline and diminish in size toward the proximal face. In *C. aglolepis* (Fig. 2 A–C) the verrucae are obscure. The verrucae of *C. aglolepis* (Fig. 2 A–C) and *C. lorentzzi* (Fig. 2 D–F) are larger than in *C. major* (Fig. 2 G–I) and *C. tucumanense* (Fig. 2 J–L).

The perispore is 0.4–1 μm thick, smooth and perforated. With LM it is aparently single-layered in section and follows the verruca contours (Fig. 2 C, E and H). Most of the species analyzed have irregularly distributed globules on

TABLE 1. Specimens studied.

Taxon	Voucher specimens
Polypodium L.	
P. argentinum Maxon	Jujuy: Dpto.Yala, *Eskuche 119* (LP), MP 3853 Salta: Dpto. Santa Victoria, *Hurrel 51* (LP), MP 3852 Tucumán: Dpto. Tafí, *Maruñak, Olivia & Puezo 345* (LP), MP 3847
P. bryopodum Maxon	Jujuy: Dpto. Tumbaya, Volcán, *Cabrera, Torres, Tur & Kiesling 18353* (LP), MP 3846 Tucumán: Dpto. Chicligasta, Estancia Santa Rosa, *Venturi 4802* (LP), MP 3851
P. chrysolepis Hook	Salta: Dpto. Orán, cerro La cueva, (SI, *1321*), MP 3913. Tucumán: Dpto Tafi, Río Potrero, *Bruchter s/n* (LP), MP 3845 Tucumán: Dpto Tafi, La Ventanita, *Castillo 35* (SI), MP 3914
P. lasiopus Klotzsch	Jujuy: Dpto. Capital, Laguna de Yala, *Palací et al. 893* (SI), MP 3884 Salta: Dpto. Guachipas, Estancia Pampa Grande, *Hawkes et al. 3976* (LP), MP 3887
P. loriceum L.	Salta: Dpto Santa Victoria, Los Toldos, *Martínez 641 et al.* (LP), MP 3886 Salta: Dpto Santa Victoria, Los Toldos, *Martínez et al. 595* (LP), MP 3957
P. pleopeltidis Fée	Jujuy: Dpto. Ledesma, *Cabrera, Kiesling & Zardini 24008* (LP), MP 3849 Jujuy: Dpto. Ledesma, Abra de las Cañas, *de la Sota 4428* (LP), MP 3917.
P. squalidum Vell.	Jujuy: Dpto. Santa Bárbara, *Zuloaga & Deginami 327* (LP), MP 3882 Jujuy: Dpto Ledesma, Calilegua, Arroyo del medio, *Cabrera et al 30363* (LP), MP 3956 Salta: Dpto Oran, Parque Nac. El Rey, Arroyo La Sala, *Brown 90* (LP), MP 3844
P. tweedianum Hook.	Jujuy: Dpto. Capital, Lozano, *Krapovickas & Schinini 35825* (LP), MP 3881 Salta: Dpto. Orán, Aguas Blancas, *Palací 104* (LP), MP 3885 Tucumán: Dpto. Monteros, Quebrada Pueblo Viejo, *de la Sota 4066* (LP), MP 3848
Campyloneurum *C. Presl*	
C. aglaolepis (Alston) de la Sota	Salta: Dpto. Capital, San Lorenzo, *Cabrera 3061* (LP), MP 3888 Salta: Dpto. Capital, Quebrada San Lorenzo, *Cabrera 9118* (LP), MP 3916
C. lorentzii (Hieron.) Ching	Salta: Dpto. Capital, Quebrada San Lorenzo, *Palací 160* (LP), MP 3889 Salta: Dpto. Capital, Parque Nacional El Rey, *Brown 983-2* (LP), MP 3954 Tucumán: Burruyacú, Los Pinos, *Borsini s/n* (LP), MP 3952 Jujuy: Dpto. Capital, Quebrada Yala, *Cabrera y Kiesling 25227* (LP), MP 3953
C. major (Hieron. *ex* Hicken) Lellinger	Jujuy: Dpto. Ledesma, Mesada de las Colmenas, *de la Sota 4483* (LP), MP 3941

TABLE 1. Continued.

Taxon	Voucher specimens
C. tucumanense (Hieron.) Ching	Jujuy: Dpto. Ledesma, 10 a 20 km de Libertador Gral. San Martín, *Krapovickas, Schinini & C. Quarín 26641* (LP), MP 3900
	Salta: Dpto. Sta. Victoria, Baritú, *Marmol, Legname & Cuezzo 8762* (LP) MP 3902
	Tucumán: Dpto. Tafí, Quebrada de Tafí, *Venturi 871* (LP), MP 3901
Pecluma M.G. Price	
P. filicula (Kaulf.) M.G. Price	Jujuy: Dpto. Valle Grande, Mesada de las Colmenas, *Fabris 3425* (LP), MP 3912
	Salta: Dpto. Orán, Aguas Blancas, Quebrada El Nogal, *Palací 92* (LP), MP 3911
P. oranense (de la Sota) de la Sota	Jujuy: Dpto. Capital, Cerro Labrado, *de la Sota 4310* (LP), MP 3909
	Salta: Dpto. Santa Victoria, *Mármol, Cuezzzo (h) & Cuezzo 9209c* (LP), MP 3910
P. venturi (de la Sota) M.G. Price	Salta: Dpto. Capital, Quebrada San Lorenzo, *Palací 166* (LP), MP 3890
	Salta: Dpto. Santa Victoria, Los Toldos, Quebrada El Astillero, *Palací 499* (LP), MP 3907
	Tucumán: Dpto. Monteros, Quebrada Pueblo, *de la Sota 4059* (LP), MP 3908
Microgramma C. Presl	
M. squamulosa (Kaulf.) de la Sota	Jujuy: Dpto. Capital, La Cuesta, *Cabrera et al. 18856* (LP), MP 3904
	Salta: Ruta Nac. 9, Pampa Grande, *Calandra s/n* (LP), MP 3903
	Tucumán: Dpto. Monteros, Quebrada de los Sosa, Casa de Piedras, *Krapovickas & Cristóbal 20456* (LP), MP 3905
Phlebodium *(R. Brown) J. Smith*	
P. pseudoaureum (Cav.) Lellinger	Jujuy: Dpto. Capital, *Cabrera 8178* (LP), MP 3906
	Salta: Dpto. Rosario de Lerma, *Venturi 8227* (LP), MP 3850
	Salta: Dpto. Orán, Aguas Blancas, *Palací 96* (LP), MP 3918
Pleopeltis *Humb. & Bonpl. ex Willd.*	
P. macrocarpa (Bory ex Willd.) Kaulf.	Salta: Dpto. Santa Victoria, Los Toldos, *Martínez et al. 653* (LP), MP 3891
	Salta: Dpto. Santa Victoria, camino a Los Toldos, *Martínez et al. 644* (LP), MP 3915

the spore surface (Fig. 2 C, D, G, J and K). In some samples (*de la Sota 4483*, LP; Fig. 2 H) small perforations were observed across the perispore surface.

The number of spores produced per sporangium was estimated in several specimens in order to check for the possiblity of apogamy in *Campyloneurum tucumanense*. In *Krapovickas et al. 26641* (LP) and in *Venturi 871* (LP), 64 spores per sporangium were estimated and, apart from mature spores, hyaline

TABLE 2. Spore morphological data of the Polypodiaceae from Northwestern Argentina (sizes in μm, mean value in parentheses).

Taxon	Major equatorial diameter	Minor equatorial diameter	Polar diameter	Laesura length	Exospore	Perispore	Exospore ornamentation
Polypodium argentinum	76.6 (86.2) 95.6	54 (61.3) 68.5	46 (54.8) 64	46 (52.7) 58.6	2.5 (3.3) 4.3	0.4 (0.8) 1	Verrucate, with globules
P. bryopodum	61.9 (75.6) 87.1	54.7 (61.9) 72	34.9 (45.2) 61.5	44.8 (55.4) 55.2	3.1 (3.9) 4.9	0.4 (0.7) 0.8	Verrucate, with globules
P. chrysolepis	71.4 (78.4) 82.1	48.1 (54.6) 61.8	38.9 (48.1) 56.2	34.4 (40.8) 46.3	1.8 (3.0) 3.4	0.4 (0.6) 0.6	Verrucate, with globules
P. lasiopus	74 (83) 94.8	43.3 (57.2) 64.1	39.9 (52.1) 59.8	30 (38) 41.5	2.1 (3.3) 4.1	0.4 (0.7) 0.9	Verrucate, with large verrucae and ridges
P. loriceum	51.6 (63.5) 74.5	37.2 (40.5) 45	30.4 (36.7) 47.8	41.5 (45.2) 49.8	2.9 (3.4) 4.2	0.4 (0.6) 0.8	Verrucate, with large verrucae and ridges
P. pleopeltidis	58.5 (66.1) 75.7	37.2 (42.1) 49.5	32.5 (42.6) 46	31.6 (34.5) 38.6	2.1 (2.9) 3.9	0.8 (1.1) 1.2	Verrucate, with.large verrucae
P. squalidum	47.8 (57.2) 64.7	35.1 (40.4) 46.7	33.3 (39.6) 45.2	22.5 (26.9) 31.2	1.8 (2.15) 2.5	0.57 (0.7) 1	Verrucate, with globules
P. tweedianum	59.5 (69.6) 80.7	41.8 (47.4) 51	30.5 (37.4) 44.4	44.8 (50.1) 58	1.7 (2.1) 2.5	0.3 (0.4) 0.6	Verrucate, with. low verrucae, and globules
Campyloneurum aglolepis	67.6 (73.8) 83	47.7 (54.9) 64.7	47.3 (51.1) 55.6	29.5 (34.6) 39.4	2.1 (2.4) 2.9	0.7 (0.8) 0.9	Verrucate, with low verrucae, and globules
C. lorentzii	64.9 (71.5) 79.1	54.8 (59.2) 63.9	41.8 (48.9) 55.9	26.0 (30.9) 39.1	2.2 (2.9) 3.3	0.5 (0.7) 0.8	Verrucate, with globules
C. major	71.4 (71.8) 72.2	39.0 (45.2) 51.5	37.3 (42.5) 45.6	44.8 (45.2) 45.6	1.5 (1.6) 1.7	0.4 (0.5) 0.8	Verrucate, with few globules
C. tucumanense	85.1 (91.0) 93.8	57.3 (63.6) 71.4	46.5 (54.3) 59.3	45.6 (53.9) 61.4	3.3 (3.7) 4.1	0.8 (0.9) 1.3	Verrucate, with few globules
Pecluma filicula	45.1 (51.9) 58	31.7 (37.7) 40.2	26.9 (32.5) 39.1	21.2 (27.1) 30.7	1.4 (1.7) 2.1	0.6 (0.8) 0.95	Verrucate, with few globules
P. oranense	44 (49.6) 54	27.5 (28.5) 33	26 (27.9) 30.5	20.5 (25.65) 30	2 (2.6) 3.4	0.5 (0.8) 1	Verrucate, with large verrucae in two levels and ridges, with globules
P. venturi	43.6 (48.7) 54	28.1 (33.1) 36.4	26.5 (30.3) 35.9	21.6 (26.31) 28	1.8 (2.14) 2.3	0.45 (0.6) 0.8	Verrucate, with large verrucae in two levels and ridges, with globules
Microgramma squamulosa	70.5 (75.9) 80.9	52.9 (55.8) 60.5	42.1 (46.6) 52.1	35.6 (38.1) 43.6	3.1 (4.1) 4.9	0.4 (0.5) 0.7	Verrucate, with few globules
Phlebodium pseudoaureum	35.6 (38.2) 41.3	21.8 (23.8) 26.7	21.1 (23.5) 25.9	19.4 (20.8) 25.1	3.2 (3.6) 4.1	0.9 (1.0) 1.2	Tuberculate, with few globules
Pleopeltis macrocarpa	74.7 (81.6) 86.3	45.6 (52.1) 64.3	77.6 (80.1) 81.7	41.5 (45.4) 50.6	1.7 (2.0) 2.3	0.9 (1.2) 1.5	Verrucate, with few globules

and small immature spores were also observed. In *Legname* & *Cuezzo* 8762 (LP) 32 spores per sporangium were estimated.

Pecluma (Table 2; Fig. 3 A–K)

This genus is represented by three species in the northwest of Argentina: *P. filicula* (Kaulf.) M. G. Price, *P. oranense* (de la Sota) de la Sota and *P. venturii* (de la Sota) M. G. Prince. *Pecluma oranense* is endemic to Salta and Jujuy, growing as an epiphyte in the basal forest and in *Podocarpus parlatorei* dominated forest. This genus is characterized by pinnatifid, pubescent lamina, sori borne at the tip of a vein and nonclathrate basifixied rhizome scales. *Pecluma filicula* is the smallest of the species studied. It reaches ca. 20 cm in length and has a pubescent and scaly rachis, whearas *P. oranenese* and *P. venturi* have longer lamina and have a pubescent, but never scaly rachis.

The spores are monolete and yellowish in *Pecluma oranense* and *P. venturi* and light-brown in *P. filicula*. They are ellipsoidal to oblong in polar view (Fig. 3 A, E, G and H), and concave and convex distally in equatorial view (Fig. 3 B, D and I). They are 44–58 μm in major equatorial diameter and 26–40 μm in polar diameter.

The exospore is 1–2 μm thick in *Pecluma filicula* and 2–3.4 μm thick in *P. oranense* and *P. venturi* . With LM it is apparently double-layered in section and with a verrucate surface. The inner layer (ie) is bright yellow and the outer one (oe) light yellow. In *P. filicula* the ie: oe ratio is 1:1 to 1:2, while in *P. oranense* and *P. venturi* the ie: io is 1:4–1:5.

The exospore of *Pecluma filicula* has heterometric, low verrucae arranged on one level (Fig. 3 A–C). When observed with LM in equatorial view, exospore thickness increases toward the proximal face. The perispore is 0.6–0.9 μm thick, verrucate and perforate. The perforations are located between verrucae (Fig. 3 C). The perispore is uniformly adhered to the exospore.

In *Pecluma oranense* (Fig. 3 D–F) and *P. venturi* (Fig. 3 G–K) the verrucae are arranged in two levels. The upper level has spheres and large verrucae, which are sometimes laterally fused. The lower level has heterometric verrucae, which are sometimes fused but are smaller than the upper ones. In both species verruca size diminishes toward the proximal pole. When observed with LM in equatorial view, exospore thickness diminishes toward the proximal pole.

As seen with LM the perispore is 0.4 to 1 μm thick, apparently single-stratified and adhered to the exospore. It has a micro-verrucate and baculate surface (more evident in *Pecluma oranense* and *P. venturi;*Fig. 3 F, J and K).

The globules in *Pecluma venturi* (Fig. 3 J) showed a micro-verrucate surface like that of the exospore.

Microgramma (Table 2; Fig. 4 A–D)

Only one species, *M. squamulosa* (Kaulf.) de la Sota, is reported for Northwest Argentina. A hybrid, *Microgramma x mortoniana*, was reported for

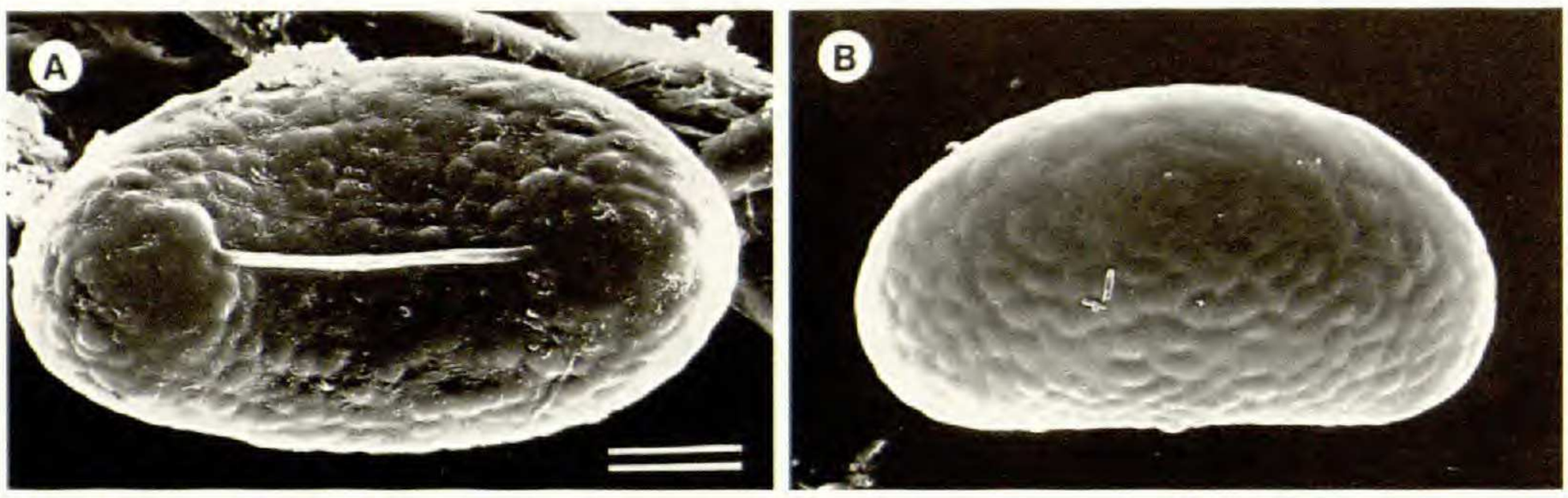

FIG. 2. SEM micrographs of *Campyloneurum* spores.
A–C, *Campyloneurum aglolepis* (*Cabrera 3061*). A. Proximal view. B. Equatorial view. C. Detail of the surface, showing single or grouped globules on the abraded perispore. D–F, *Campyloneurum lorentzii* (*Kiesling 25227*). D. Proximal view, isolated globules are present on the surface. E. Distal surface in detail, the sculpture is slightly verrucate. F. Equatorial view, the verrucae are polyedric

Salta by de la Sota, *et al.* (1999). The hybrid was not included in this study because the specimen was sterile.

The spores of *Microgramma squamulosa* are monolete, yellowish to light brown, ellipsoidal or oblong in polar view (Figs. 4 A, B) and plane/concave-convex in equatorial view, 70–80 μm in major equatorial diameter and 42–52 μm in polar diameter.

The exospore is 3–5 μm thick and apparently double-layered in section when observed with LM. The inner layer (ie) is bright yellow and the outer (oe) light brown. The ie: oe ratio varies from 1:4 to 1:6. The exospore sculpture is verrucate. The verrucae have a micro-verrucate surface (Fig. 4 C–D). The verrucae are single, or fused to form ridges (Fig. 4 A–D) which diminish in size toward the proximal face (Fig. 4 A–B).

The perispore is 0.4–0.7 μm thick, apparently single-layered in section with the LM closely adhering to the exospore and smooth or micro-verrucate with perforations (Fig. 4 C–D). Occasionally, globules are observed on its surface (Fig. 4 C–D).

In spores of *Krapovickas & C.L. Cristobal* 20456 (LP) granular material was observed on the surface (Fig. 4 C).

Phlebodium (Table 2; Fig. 4 E–I)

One species of this genus, *P. pseudoaureum* (Cav.) Lellinger, is present in the northwest of Argentina. It grows as a deciduous epiphyte in the basal forest. The laminae are large, pinnatifid and glabrous with anastomosing venation.

The spores are monolete, yellowish, oblong to ellipsoidal in polar view (Fig. 4 E and G) and plane to concave and convex distally in equatorial view (Fig. 4 F). Their diemnsions are 35–42 μm in major equatorial diameter and 21–26 μm in polar diameter.

The exospore is 3–4 μm thick, at LM apparently double-layered in section, the inner layer (ie) being lighter than the outer layer (oe). The ratio ie: io is 1:3 to 1:5. It is compact as seen in fractures observed with SEM (Fig. 4 I). The exospore is tuberculate (Fig. 4 E–H) and the tubercles are single or fused, with a blunt or truncate apex. They seem to be formed by coalescent roads (Fig. 4 F–H).

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in shape and heterometric, the size of the verrucae diminish toward the proximal pole at both sides of the lesura. G–I, *Campyloneurum major* (*de la Sota 4483*). G. Equatorial view, the verrucae are round, small, and densely packed. H. Distal surface in detail. Irregularly distributed perforations (arrow head) are present and some verrucae are fused. I. Fracture through the sporoderm. J–L, *Campyloneurum tucumanense*. (J and L: *Schinini & C. Quarín 26641*; K: *Legname & Cuezzo 8762*). J. Distal view, globules are occasionally present. K. Equatorial view. Verruca size diminishes toward the proximal pole which is at the bottom. L Detail of the distal surface, the verrucae are heteromorphic and heterometric and some of them fused. Their surfaces are slightly verrucate. Scale bars: A, B, D, F, G, J and K 10 μm; C, E, H and L: 5 μm; I: 2 μm.; e: exospore, p: perispore.

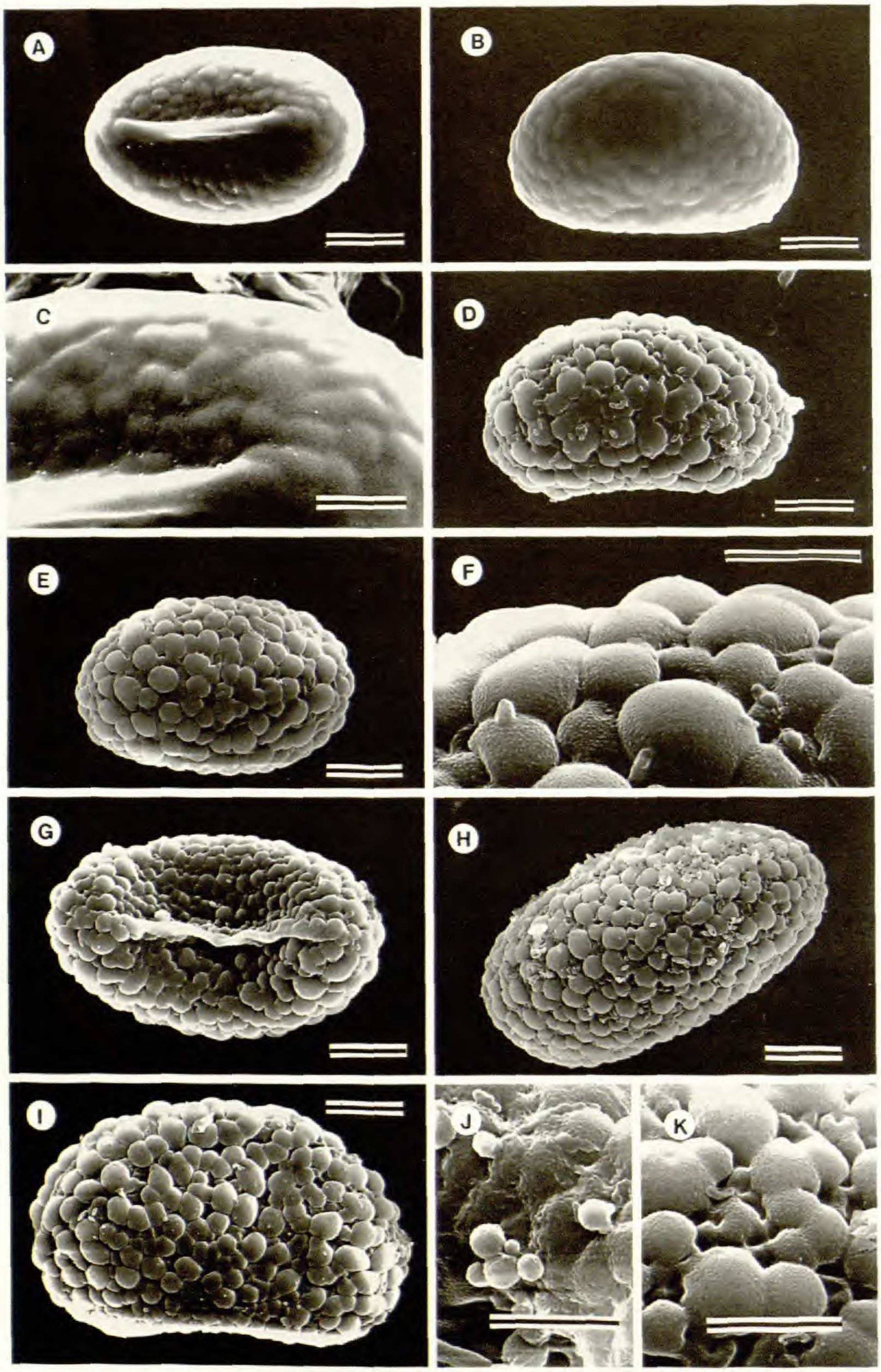

FIG. 3. SEM micrographs of *Pecluma* spores.
A–C, *Pecluma filicula* (*Palací 92*), A Proximal view. Verruca size diminishes toward the proximal pole. The laesura ridge is thick. B. Equatorial view. C. Detail of the proximal surface; verruca surface is smooth and perforations are present between verrucae at the sides of the laesura (arrowhead). D–F, *Pecluma oranense* (*de la Sota 4310*) D. Equatorial view, showing verruca

The perispore is up to 1 μm thick, with LM it is apparently single-layered in section, smooth with few perforations (Fig. 4 H) and adherant to the exospore.

Pleopeltis (Table 2; Fig. 4 J–M)

One species, *P. macrocarpa* (Bory ex Willd.) Kaulf., is reported (Ponce, 1996) for the northwest of Argentina. It grows as an epiphyte or is epipetric in the basal forest between 1000 and 2000 a. s. l. It is characterized by a scarcely pubescent lamina, ellipsoidal sori, and peltate scaly paraphyses.

The spores are monolete, light brown, ellipsoidal to sub-spheroidal in polar view (Fig. 4 J) and plane/concave-convex in equatorial view (Fig. 4 K), 75–86 μm in equatorial diameter and 46–64 μm in polar diameter.

The exospore is 1.7–2.3 μm thick and verrucate. The verrucae are very low, isolated, and have a micro-verrucate surface (Fig. 4 J–L). With LM it is apparently double-layered in section, the inner layer (ie) being brighter than the outer layer (oe). The ie: oe ratio is 1:3–1:4. The exospore is apparently compact, as seen in fractures with SEM (Fig. 4 M).

The perispore is 1 μm thick, and apparently single-stratified with LM and adhered to the exospore. Its surface is smooth or micro-verrucate and perforated (Fig. 4 L). Globules were observed on the surface (Fig. 4 K). In some specimens (*Martínez et al. 644,* LP), there were abundant perforations distributed on the whole surface of the perispore (Fig. 4 L).

Our material of *Pleopeltis macrocarpa* has spores with characteristics similar to those described by Tryon & Tryon (1982) based on material from Peru. These authors illustrated the spores of *P. macrocarpa* with SEM and described the surface as verrucate, with globules and ca. 70 μm.

Polypodium (Table 2; Figs. 5, 6)

Eight species of *Polypodium* grow in the northwest of Argentina. They are *P. argentinum* Maxon, *P. bryopodum* Maxon, *P. chrysolepis* Hook., *P. lasiopus* Klotzsch, *P. loriceum* L., *P. pleopeltidis* Fée, *P. squalidum* Vell, and *P. tweedianum* Hook. These species are epiphytic or rupestral, rarely terrestrial, and are characterized by a pinnatifid lamina, with scales or glandular hairs and with sori borne at the tip of a vein.

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diameter diminishes toward the proximal pole. E. Distal view, showing verrucae at different levels; some of the verrucae are heterometric and fused. F. Magnification of the distal surface of the spore in picture 4; verruca surfaces are micro-verrucate and the verrucae are laterally fused. G–K, *Pecluma venturii* (de *la Sota 4059*) G. Proximal view, the verrucae are smaller here than in the rest of the spore surface and the laesura is membranaceous and high. H. Distal view showing verrucae at different levels with some of them fused. I. Equatorial view, the verrucae are so densely packed that most of them have a polygonal outline, and some are fused. Verrucae diameter diminishes toward the proximal pole. J and K. Magnifications of the surface, showing micro-verrucation of surface. In J, isolated and grouped globules are present on their surfaces; note the similarity between globule and verruca surface. Scale bars: A, B, D, E, G, H, I: 10 μm; C, F, K and J: 5 μm.

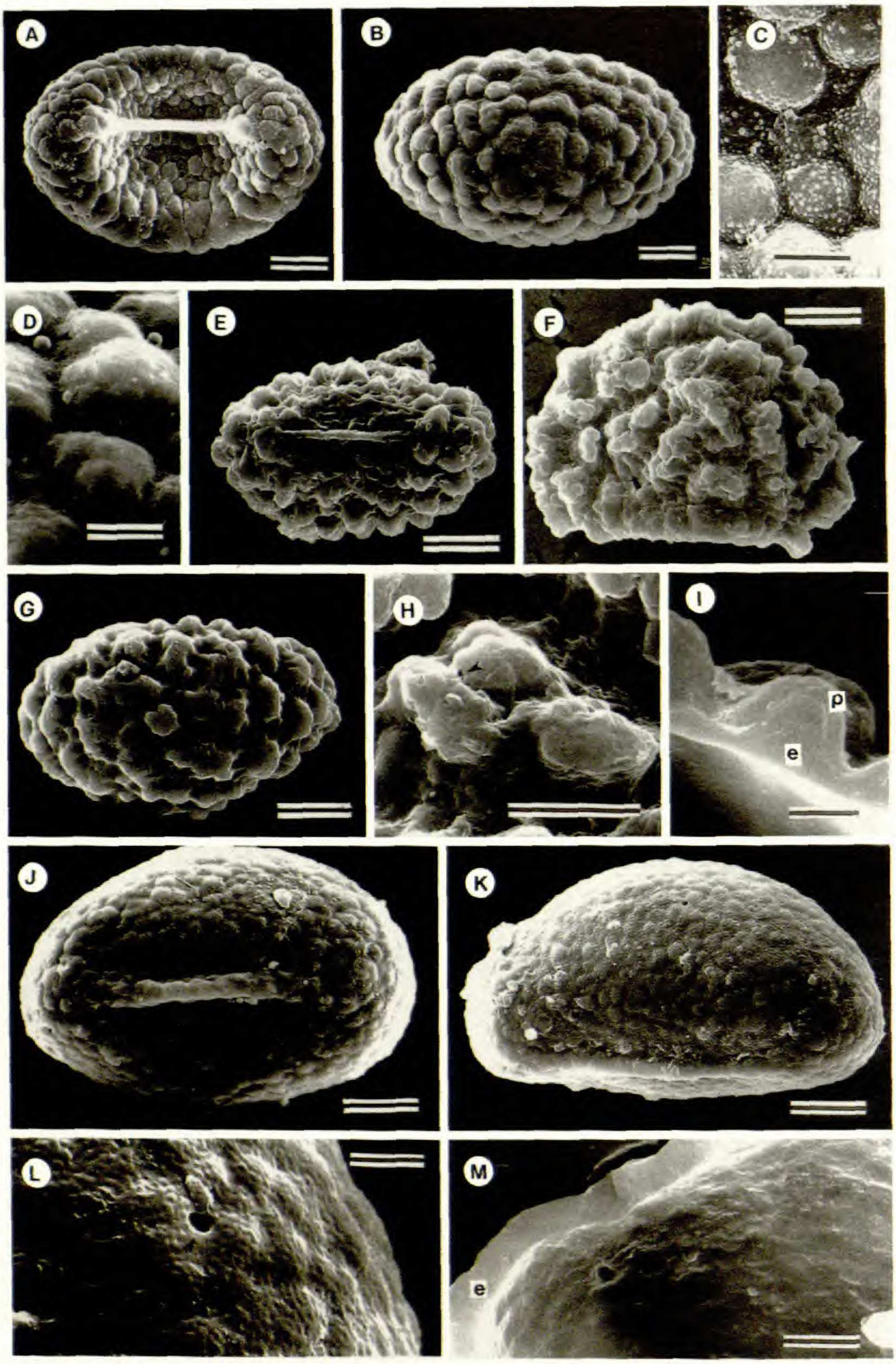

FIG. 4. SEM micrographs of *Microgramma, Phlebodium* and *Pleopeltis* spores.
A–D, *Microgramma squamulosa* (*Krapovickas & Cristobal 20456*) A. Proximal view, the verrucae are smaller on both sides of the laesura. B. Distal view, the verrucae are both irregularly-shaped and irregularly-sized. Some of them are fused, forming ridges. C. Detail of the proximal surface of the spore in A with polygonal verrucae and micro-verrucate surface. Small globules and granular

The spores are monolete, light brown to yellowish, ellipsoidal to oblong in polar view (Fig. 5 A, D, G, and L; Fig. 6 B, C and F) and plane-convex to plane-hemispherical in equatorial view (Fig. 5 B, E, H, K and N; Fig. 6 A, E, H and I), 52–98 μm in equatorial diameter and 29–65 μm in polar diameter.

The exospore is generally 2–4 μm thick, but range to 8 μm thick *in Polypodium lasiopus*, and verrucate. As seen with LM it is apparently double-layered in section and in equatorial view it increases in thickness toward the proximal face. Variation in size and degree of verruca fusion were observed in different specimens.

In all the species the perispore is up to 1 μm thick. With LM it is apparently single-layered in section and smooth, rugulate or micro-verrucate according to the species (Fig. 5 C, F, I, J, M and O; Fig. 6 D, G and J).

There are perforations on the exospore and perispore surfaces in all the analized species, located on and between verrucae (Fig. 5 C and F; Fig. 6 D and J)

Sporopollenin globules either single or associated in masses are adherant to the perispore in most of the species analized. They differ in size, number and distribution. These globules show a structure similar to that of the sporoderm (Fig. 5 B–E, G, H, J–K; Fig. 6 A–C, E–J).

In *Polypodium argentinum* the verrucae are uniform in shape and size, and isoleted or grouped globules are adherant to the perispore (Fig. 5 A–C). The spores of *Polypodium chrysolepis* (Fig. 5 G–J) have low verrucae. The verrucae in *Polypodium lasiopus* (Fig. 5 K–M) and *P. loriceum* (Fig. 5 N–O) are circular to polyhedral and fused, to form radial ridges across the proximal face. The spores of *P. squalidum* are the smallest within the Polypodiacea studied here, the verrucae are uniform in size and shape, and globules are adherant to the perforated perispore (Fig. 6 E–G). The spores of *Polypodium tweedianum* (Fig. 6 H–J) have verrucae that are perforated, polyhedral and variable in size.

According to observations with LM, the globules of *Polypodium bryopodum* (Fig. 5 D–F) and *P. pleopeltidis* (Fig. 6 A–D) show a central zone much dense

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material are abundant on the whole surface. D. Detail of the surface of the spore in B showing micro-verrucate verruca surface and scattered globules (arrow head) of different sizes are present on and between the larger verrucae. E–I, *Phlebodium pseudoareum* (*Cabrera 8178*). E. Proximal view, the elements of the sculpture are low on either side of the laesura; the sculpture is tuberculate. F. Equatorial view, showing fusion of several basal ridges (arrow) to form tubercles. G. Distal view; continuity between basal ridges of continuous tubercules is evident (arrow heads). H. Detail of the equatorial surface with several large, grouped tubercles. Several ridges form the bases of the grouped elements and the spaces between tubercles are deep; perforations indicated by arrow head. I. Fracture across the sporoderm that exposing the juncture between exospore and perispore. J–M, *Pleopeltis macrocarpa* (*Martínez et al. 644*). J. Proximal view. The laesura is a short and the sculpture is verrucate; the verrucae are smaller on the sides of the laesura. K. Equatorial view. The verrucae are low and mainly rounded; their surfaces are micro-sculptured. Globules of different sizes are fused to the perispore surface and are more evident on the left. L. Detail of the equatorial surface showing obscure verrucae shape due to the thick perispore; the perispore surface is micro-verrucate and perforated (arrow head). M. Fracture across the sporoderm. A different zone in the exospore toward its inner surface can be appreciated. Scale bars: A, B, E, F, G, J, K: 10 μm; C, D, H, L and M: 3 μm; I: 2 μm

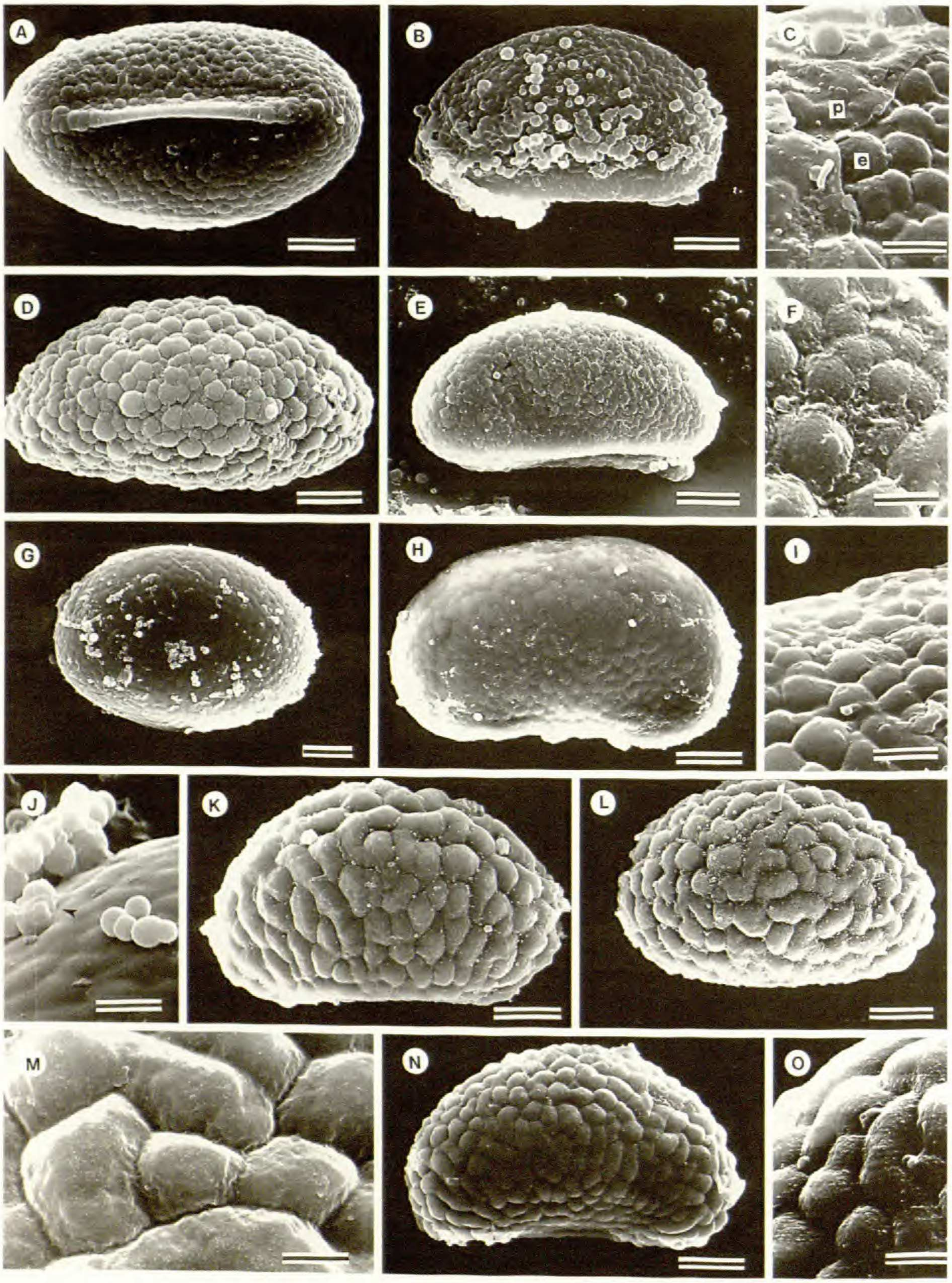

FIG. 5. SEM micrographs of *Polypodium* spores.
A–C, *Polypodium argentinum* (*Hurrel 51*) A. Proximal view, the laesura is a straight, thick ridge. The size and shape of ornamentation elements are uniform. B. Equatorial view, showing numerous globules attached to the perispore surface. C. The surface in detail with abraded perispore evident on the left and scattered globules of varied sizes fused to the perispore surface. Perforations are also

and so could be classified as "captive type" (sensu Lugardon, 1981). When observed with SEM, these globules seem to be sculptural elements. (Fig. 5 F; Fig. 6 D). In both species exospore ornamentation is verrucate. The verrucae are heterometric and located on a lower level than the globules. (Fig. 5 F; Fig. 6 D)

Discussion and Conclusions

The large spore size in *Campyloneurum tucumanense*, together with 32 or 64 spores per sporangium, and the greater plant size are characteristics probably related to polyploidy (Tryon & Lugardon, 1991). Walker (1985) detected polyploids in Jamaica and diploids among the South American species of this genus. Cytological studies are needed in order to determine if *C. tucumanense*, apparently endemic to the northwest of Argentina, is a polyploid.

Variations from 49 μm up to 95 μm in spore size were observed within the genus *Polypodium*. According to Tryon & Tryon (1982), the *Polypodium* species from America are diploid or tetraploid with relatively stable chromosome numbers. Further cytological studies of the material from Argentina would explain if the size differences are associated with differences in ploidy levels.

Phlebodium pseudoaureum has the smallest spores among the Polypodiaceae analyzed in this work and its tuberculate exospore is the thickest. These features, together with its greater plant size and pinnatifid glabrous lamina with anastomosing venation, differentiate it from other species in the study area.

The general features of the exospore in the species analyzed are in agreement with those of the "Polypodium vulgare" type as described by Hennipman (1990).

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present on the perispore (arrow head). The exospore is exposed on the right. The verrucae are different in size and shape, laterally fused and relatively smooth surface. Perforations are evident mostly at the junctions between the verrucae. D–F, *Polypodium bryopodum* (D: *Venturi 4802*, E–F: *Tur & Kiesling 18353*) D. Distal view with verrucate ornamentation. The verrucae are heterometric, mainly poligonal in outline, densely packed and laterally fused. E. Equatorial view with globules on the perispore (arrow head). F. Detail of a spot of the spore in figure E showing a micro-verrucate surface of the verrucae and perforations. G–J, *Polypodium chrysolepis* (*Castillo 35*) G. Distal view with single or grouped globules off different sizes. H. Equatorial view, the verrucae are low and apparently the perispore constitute a thin cover that makes their shapes slightly evident. There are a few scattered globules attached to the surface. I and J. Surface details. In I the characteristic of the verrucae are more evident than in J. J. masses of globules on the perispore surface, some of them are fused to the perispore (arrow head). The perispore surface is fairly smooth. K–M, *Polypodium lasiopus* (*Hawkes et al. 3976*) K. Equatorial view, the verrucae are tangentially elongated at the equatorial zone, forming true ridges. Small granules are present mainly at the junction places. L. Distal view, granules are present between sculpture elements. M. Detail of the surface showing irregular sizes and polygonal verrucae, mostly fused forming ridges. The perispore surface seems to be fairly smooth to scabrate. N–O, *Polypodium loriceum* (*Martínez* 641 *et al.*). N. Equatorial view, the verrucae are numerous per area unit, round in shape and low, but there is a general tendency to verruca fusion in the form of ridges. O. Detail of the surface, showing fused verrucae in the form of ridges. The perispore surface is rugulate. Small-sized globules are attached to the perispore surface in some places (arrow). Scale bars: A, B, D, E, G, H , K, L, N: 10μm; C, F, I, J, M and O: 3 μm.

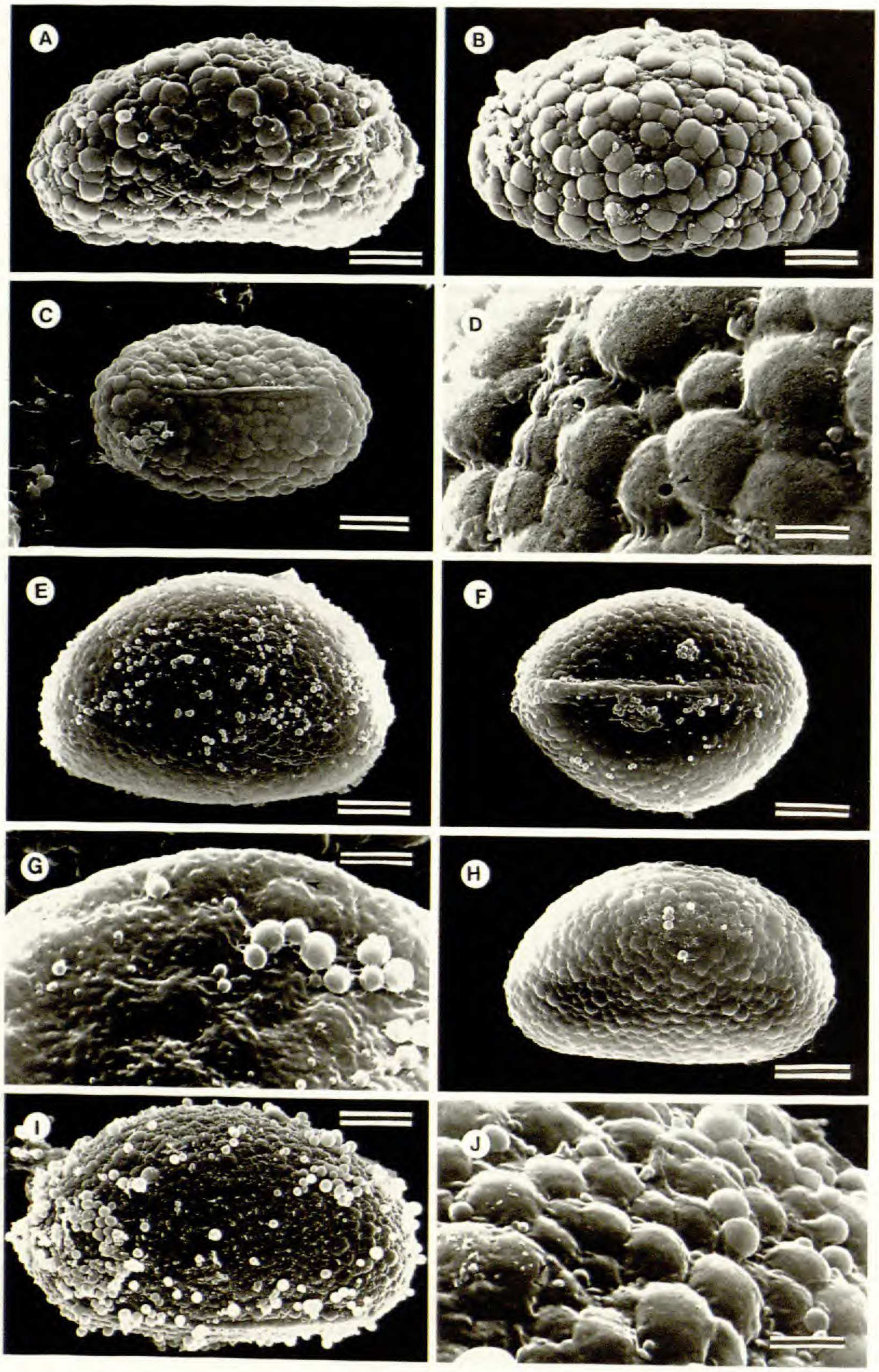

FIG. 6. SEM micrographs of *Polypodium* spores.
A–D, *Polypodium pleopeltidis* (*Cabrera, Kiesling & Zardini* 24008). A. Equatorial view, the verrucae are smaller toward the proximal pole. Scattered globules can be appreciated at different places B. Distal view, the surface is uneven and it has verrucae of different sizes and shapes in different levels. The large verrucae are laterally fused but each of them keeps the original outline.

Variations in sporoderm thickness, size of sculptural elements, number of perforations, and presence of granular material on spore surfaces were not considered to be as diagnostic characteristics because, they may be related to different stages of development (van Uffelen, 1992).

Perforations observed in the perispore and exospore of the analyzed spores could be associate with microchannels traversins the exospore; such structures were described by Lugardon (1974), van Uffelen (1992) and Hennipman (1990). The latter author classified the channels according to their size, shape and location within the exospore.

In all of the analyzed taxa the spore surface is covered with irregularly distributed globular bodies with a smooth or micro-verrucate surface, according to the taxa. These globules were acetolysis resistent and showed variable densities among spores of the same taxa. With LM they showed a density similar to that of the sporoderm. In some samples, *Polypodium bryopodum* and *P. pleopeltidis*, these globules were mistaken for elements of the exospore ornamentation. According to Lugardon (1981), these sporopollenin globules are usually present in the Filicopsids, and they were described as having a smooth surface and the same structure as the sporoderm. The globules were also analyzed using TEM in other taxa of Polypodiaceae (van Uffelen & Hennipman, 1985; Hennipman, 1990; van Uffelen; 1993 and Tryon & Lugardon, 1991).

We would noted in our analysis that the spores of some *Polypodium* species from the study area (*P. argentinum*, *P. squalidum* and *P. chrysolepis*) have similarities to those of *Pleopeltis*, some others (*P. bryopodum* and *P. pleopeltidis*) are similar to those of *Pecluma*, and others, such as *P. lasiopus* and *P. loriceum*, to those of *Microgramma*. These observations are in agreement with de la Sota (1977), who suggested that *Polypodium* is not a well delimited genus, and that certain taxa are closer to *Microgramma* whearas some others are closer to *Pleopeltis*. Later systematic studies grouped several species of *Polypodium* within *Pecluma* (Price, 1983), while others species were transferred to *Pleopeltis* (de la Sota, *in press*) and the species *Polypodium*

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Small, single globules attached to the surface can be seen in several places (arrow head). C. Proximal view. The laesura is marked by a straight ridge. The verrucae are small on both sides of the laesura. D: Detail of the surface. The verrucae are heterometric and the spaces between them are masked possibly by the perispore. Perforations of different sizes are located mainly on and between the verrucae (arrow heads). The perispore is micro-verrucate. E–G, *Polypodium squalidum* (*Zuloaga & Deginami 327*). E. Equatorial view, the verrucae are numerous per area unit, uniform in shape and size. Scattered single and grouped globules are attached to the surface. F. Proximal view. The laesura appears as a stick ridge. There are also globules at the proximal pole. G. Detail of the surface. The limits between the verrucae of the exospore below are obscure. The perispore surface is rugate and perforations are evident (arrow heads). Single and grouped globules of different sizes with a smooth surface are fused to the perispore. H–J, *Polypodium tweedianum* (*Palací 104*). H and I. Equatorial views. The presence of globules is variable. Some verrucae are fused, forming short ridges. J. Detail of surface. Verrucae are clearly defined although laterally fused. Globules of different sizes are fused to the surface. The perispore surface is relatively smooth, although some perforations are present (arrow heads). Scale bars: A, B, C, E, F, H, I: 10 μm; G, D and J: 2 μm.

chrysolepis was considered as *Microgramma* by Tryon & Stolze 1993 based on some venation characteristics.

The spores of *Polypodium loriceum* and *P. lasiopus* differ from other studied *Polypodium* species in exospore ornamentation. In the former species the verrucae are generally fused and form radial ridges proximally. These species belong to the "*Polypodium-loriceum* group" that is represented by three species in Argentina (de la Sota, Giudice & Gaute, *pers.comm.*). Our observations suggest that spores are systematic of value at infrageneric level.

Within *Pecluma*, *P. fillicula* is differentiated from other species on the basis the small size and a pubescent-scaly rachis. The thin exospore, low verrucae and smooth perispore allow it to be differentiated from others species within the genus.

Concluding Remarks

Five of the genera of Polypodiaceae from Northwestern Argentina, *Campyloneurum*, *Microgramma*, *Pecluma*, *Pleopeltis* and *Polypodium,* have verrucate spores, whereas the spores produced by *Phlebodium* are tuberculate. Within the verrucate spores taxa, variations were observed in verrucae size, shape, surface pattern and degree of fusion. The large size, together with other characteristics of *Campyloneurum tucumanenese* spores, may be related to polyploidy. In all the taxa studied the exospore was the thickest wall layer. As revealed by LM it is apparently double-layered and shows wide variation in ornamentation and structure. The inner exospore layer is thin, generally 1/3 to 1/6 the thickness of the outer layer. In acetolyzed material, as observed with LM, the outer exospore appears lighter than the inner one and, apparently, the verrucae or tubercles are restricted to the outer exospore layer.

In all cases the perispore is difficult to distinguish and measure using LM because it is thin and adhered to the exospore. However, it was possible to distinguish it in fractured spores with SEM. It is micro-ornamented, generally perforated and covered without any modification to exospore ornamentation.

The presence of sporopollenin globules is frequent on most of the spores analyzed. They are single or grouped and are either attached or fused to the perispore surface.

We noticed that there is little systematic agreement between systematists and palynologists when considering the significance of spore characteristics in the Polypodiaceae. Nevertheless, in the taxa analyzed here, palynological characteristics, together with morphological data, allow us to identify some genera such as *Pecluma* and *Phlebodium*, as well as recognize species groups within the genus *Polypodium*, e.g. the *Polypodium-loriceum* group.

We are continuing this study on the spore wall of the Polypodiaceae with TEM analyses to determine the structure and stratification of the spore wall, the relationship between perforations and channels within the exospore, and to characterize the globules.

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